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(54) Title: CURRENTS IN NARROW PORES

(57) Abstract

Current fluctuations typical of those observed with biological channels which are ion selective and inhibited by divalent cations and protons have been observed in synthetic membranes, and a process for controlling permeability of a synthetic membrane to ions and uncharged molecules may be used in a switch or sensor, for example to monitor the content of a solution, to trigger macroscopic events by local microscopic changes and to alter the ionic content of a solution e.g. desalination.

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CURRENTS IN NARROW PORES

The present invention relates to a process for
controlling the permeability to ions or uncharged molecules
of a synthetic membrane, and to switching and sensor

5   devices which use this effect.

The characteristic feature of ion channels in
biological membranes is that when an electric potential is
applied across the membrane, the resultant current
fluctuates between a conducting and a non-conducting state

10   [1]. To date most explanations of this phenomenon have been
based on the notion that conducting and non-conducting
states represent "open" and "closed" configurations of the
channel, and that the transition between them results from
the physical movement of part of the channel structure, -

15   the opening and closing of some kind of "gate" [2].

The applicants have found that current fluctuations
typical of those observed with biological channels can be
observed with purely synthetic membrane filters that may be
devoid of any added lipid or protein. Two other features

20   of biological ion channels are ion selectivity and
inhibition by divalent cations and protons [2]; these too
are displayed when current across synthetic membrane
filters is measured. It is believed that the results may
be interpreted in terms of the special properties of ion

25   conductance through water that is confined near an
interface; they may be observed whenever the contribution

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of bulk conductance is minimal, such as in a narrow pore
having a high surface to volume ratio.

Flow of electrolytes or non-electrolytes through
pores in biological membranes that are induced by agents as
5   varied as certain haemolytic viruses, bacterial or animal
toxins, immune molecules or detergents at sub-lytic
concentration show properties that are remarkably similar
from agent to agent. These include inhibition by divalent
cations, with relative efficacy $Zn^{2+} > Ca^{2+} > Mg^{2+}$ and, when
10 the agents are incorporated into planar lipid bilayers with
an applied voltage across them, fluctuations in current
typical of "single channel" openings and closings of
endogenous ion channels; in several of these systems
protons also inhibit flow (with relative efficacy $H^+ >$
15 Zn^{2+}).

The applicants have studied flow (i.e. current) of
non-electrolytes and electrolytes through purely synthetic
membrane filters and find the same effect of divalent
cations and protons, provided the membrane pores are narrow
20 enough. It is concluded that these effects are features of
flow along any liquid - solid interface, that can be
observed whenever the contribution of bulk is minimised (as
in the case of very narrow pores).

The word "pore" in the context of the present
25 invention is intended to include a hole through a membrane
or the space between two apposed surfaces that may be two
solids or two lipid monolayers.

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For simplicity the term "membrane" is used in the present description, but it is to be understood that, unless otherwise specified, this term encompasses films or sheets of any shape and thickness and whose thickness may

5 or may not be uniform, and also encompasses the combination of two solids or two lipid monolayers whose relative configuration forms at least one pore as defined above.

The possible applications of a switching device based on synthetic organic materials are wide ranging. Some are

10 listed below:

(1)   a switched pore can control the transfer of particular solvent and solute molecules across an otherwise impermeable membrane which has applications in chemical engineering.

15   (2)   a pore that may be switched from low to high conductance can also serve as a sensitive detector of particular conditions such as pH and ionic strength or even an elevation in the concentration of a particular solute molecule. Such a detector may have a very high gain (or

20 amplification) in that the concentration change required to switch the pore conductance may be very much smaller than the detected flow through the pore when it is opened. Applications of this effect include chemical and medical monitoring of solutions with the rapid detection of changes

25 in specific constituents of the solution.

(3)   A pore is switched from high to low conductance by the particular conditions that prevail within the pore

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or in the immediate vicinity of its two ends. Thus by

special preparation of this very local environment, for

example by introducing specific chemical entities in these

regions, the macroscopic bulk flow through the pore is

5   controlled by changes in the chemical environment of these

microscopic regions. This again renders it possible for

macroscopic events to be triggered by extremely local

changes which will have applications in process control.

(4)   As in semiconductor physics, once a switch has

10   been developed, then amplifying devices with controlled

gain are obtained by the application of negative feedback

around the switch and oscillators are obtained by the

application of positive feedback around the switch. The

rapidity of the switch leads to the possibility of high

15   frequency devices.

(5)   Since flow of ions is more sensitive to

inhibition by divalent cations and protons than is flow of

water, the membrane may be used to alter the ionic content

of a solution an important application in this respect is

20   in desalination processes.

The present invention provides a process for

controlling permeability to ions and uncharged molecules

through a pore as defined herein having discrete states of

differing permeability and being of less than 10 nanometers

25   radius or 20 nanometers width, which process comprises

placing the pore between a first solution containing an ion

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(the transport ion), or uncharged molecule capable of passing through the pore, and a second solution;

and controlling the permeability of ions or uncharged molecules through the pore by means of the transport ion

5   concentration in the first solution and/or the proton or multivalent ion concentration in the first and/or second solution.

Thus the invention also includes a process in which the laminar flow of ions or uncharged molecules between two

10   apposed membranes, separated by < 20 nm, may be controlled by the proton or multivalent ion concentration as described above.

The present invention also provides a switch for ionic current comprising a pore of less than 10 nm radius

15   through a synthetic membrane film or comprising two apposed synthetic membranes less than 20 nm apart forming a pore between the membranes, said pore being simultaneously in contact with a first ionic solution containing an ion capable of being transported through the membrane pore, the

20   transport ion, and with a second ionic solution,

such that when the switch is placed in an electric circuit, flow of ionic current though the pore is controlled by transport ion concentration in the first ionic solution and/or the proton or multivalent ion

25   concentration in one or both of the said ionic solutions.

The present invention also provides a sensor for sensing a parameter in a solution such as pH, ionic

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strength or solute concentration, said sensor comprising a
pore of less than 10 nanometers radius through a synthetic
membrane or comprising two apposed synthetic membranes less
than 20 nm apart forming a pore between the membranes,

5   such that when the pore is placed in contact with the
solution whose parameter is to be detected, the ionic
current passing through the pore is indicative of the
presence, absence or level of the parameter to be detected.

The term "ionic solution" in this context refers to a
10   solution containing ions which may be e.g. a solution of an
ionic material, such as a salt, dissolved in an appropriate
solvent, or may be simply a liquid, such as water, in which
a proportion of the liquid exists in the form of ions.

Suitable ions which may be transported through the
15   membrane pore include alkali metal ions (e.g. Li^+, Na^+, K^+,
Rb^+), halide ions (e.g. F^-, Cl^-, Br^-, I^-) and multivalent
ions (e.g. Mg^{2+}, Ca^{2+}, SO_4^{2-}, phosphate). Preferred ions
will depend on the use to which the process is being
applied e.g. desalination.

20   Suitable solute molecules which may be transported
include water soluble molecules whose effective size is
less than the effective cross-sectional area or width of
the pore (e.g. sugars such as glucose, amino acids, certain
hormones, neurotransmitters, drugs, immune molecules or
25   polymers such as polyethylene glycol).

Current switching behaviour typical of that observed
in an ionic channel in a naturally occurring membrane may

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be reproduced by a synthetic membrane containing narrow

pores.   The membrane may be a plastic film such as a

polycarbonate or other polyester, e.g. polyethylene

terephthalate (PETP). The applicants have investigated PETP

5   films of 5 or 10 micrometers thickness and found them to

display the effect of the invention, but thinner or thicker

films could be used, depending on the requirements of the

use to which the process is being applied. For example, the

minimum thickness may depend *inter alia* on physical

10   characteristics such as the ability of the film to be

handled by the manufacturer or user and on the pressure the

film is subject to during use. For electrical devices

(e.g. sensors) thin films are likely to prove optimal. For

flow devices (e.g. desalination) thick films are likely to

15   prove optimal; alternatively laminar flow between two

apposed membranes may be used.

The applicants believe that other synthetic membranes

exposed to the track-etch procedure described below are

also suitable for use in the present process; these include

20   polymers that are positively charged as well as those like

PETP that are negatively charged after track etching. In

the case of positively charged membranes, some of the

properties e.g. selectivity and inhibition by protons can

be reversed.

25   Narrow pores in plastic films such as polyester, e.g.

PETP, may be formed by irradiating the film with heavy ions

such as ^{132}Xe, ^{129}Xe, ^{84}Kr or ^{59}Co accelerated in a cyclotron,

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to produce tracks; the polymer is then treated with a hot

alkali [3] to hydrolyse parts of the polymer thereby

causing local solubilization of the film material

particularly in the regions affected by the heavy ion

5   irradiation.   The whole process is called track-etching.

In the case of laminar flow between two apposed

membranes track-etching of the membrane in order to create

narrow pores is unnecessary, but chemical treatment e.g.

etching alone of the surfaces may be required.

10   In the track-etching process cylindrical or conical

pores are formed which penetrate the film.   The pore

diameter depends on the energy and species of heavy ions

used for the film irradiation, on the type of polymer used

and especially on the conditions of the etching process.

15   To achieve membranes which may display the effect of

the present invention, beams of heavy ions may be used

which typically have the following energies: ^{132}Xe(121 MeV),

^{129}Xe(124 MeV), ^{84}Kr(74 MeV) and ^{59}Co(68 MeV), with an ion

fluence of from 6 x 10^{5} to 1 x 10^{11} cm^{-2}.   Appropriate

20   alkalis and temperatures for the use in this method would

be evident to the ordinarily skilled person, and for

materials such as PETP include e.g. 0.1 M NaOH, 1.0M K_2CO_3

and 2.5 M K_2CO_3 used at a temperature of about 80°C.

For the purposes of the present invention the pore

25   diameter or width should be less than 20 nanometres.

The chemical etching of pores into a material such as

polyethylene terephthalate results in hydrolysis of the

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PETP generating free carboxyl groups making the pores in
the membrane more hydrophilic, but other possible membrane
materials could be used.

The density of pores in the membranes that the

5   applicants have studied so far range from 1 to 5 x 10^9
pores per cm^2 but there is no reason to suppose that higher
pore densities could not also be used. The properties of
individual pores are independent of pore density. For
electrical devices (e.g. sensors) the pore density should

10   be low. For flow devices (e.g. desalination) the pore
density should be high; an alternative mode in the latter
case is for flow to be between two closely apposed
membranes such that the space between them is less than 20
nm.

15   Transfer of ions, i.e. ion current across such
membranes may exhibit rapid switching from a high
conductivity to a low conductivity state, even when the
voltage across the membrane is held fixed. The rapid
switching shown by such a narrow pore membrane resembles

20   the rapid switching shown by biological ion channels. This
switching may be observed whenever the ratio of surface
area to volume of the conducting phase is high, such as in
narrow pores or in the space between two closely apposed
solid surfaces or lipid monolayers. Switching can be

25   modified and controlled by controlling the amount of
particular ions such as protons and multivalent ions in
solution. This control may be achieved by adding protons

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or a source of multivalent ions, and may be reversed by
adding alkali to remove such protons or a sequestrant such
as ethylene diamine tetraacetic acid (EDTA) to remove
multivalent ions. The control of conduction through the
5   narrow pore includes control of the transfer of water and
uncharged molecules as well as ions.

The following examples illustrate the invention.

EXAMPLE 1

The filters used were approximately 5 μm thick and
10   made of polyethylene terephthalate (PETP; "Lavsan"). After
heavy ion bombardment to induce nuclear tracks, the filters
were "etched" with hot alkali; this treatment breaks
(hydrolyses) some of the ester bonds generating free
carboxyl groups. These may be negatively charged and make
15   the filters more hydrophilic [3]. Two separate filters,
having slightly different apparent pore sizes were used.
They are referred to throughout as filter 1 and filter 2.
The pore density for each filter was approximately 1
pore/cm^2.

20   Filters were clamped between two small chambers
(volume 0.3 ml each) which contained KCl (0.1 - 3M),
buffered with 0.005M Hepes pH 7.4, respectively. Current
was measured at room temperature with Ag/AgCl electrodes
monitored with a virtual grounded operational amplifier
25   with a feed-back resistor of 10^9 ohms.

The membrane switches between a high conducting or
"on" state and a low conducting or "off" state. The

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reversal potential, i.e. the sign and magnitude of the
voltage (Ψ) at zero current was used to deduce the
selectivity of the filters for cations over anions, in the
presence of a five-fold gradient of KCl. The modal current

5   in the high conducting state is obtained from amplitude
histograms as described hereafter. In Figure 1A this
current is plotted as a function of applied potential for
filter 1($\square$) and filter 2($\bullet$). The selectivity (t_+) was
calculated from the reversal potential (Ψ) using the

10 equation (I):

$$t_+ = \tfrac{1}{2}\left(1 + \frac{\Psi}{\dfrac{RT}{F} \ln \dfrac{[K^+]\ trans}{[K^+]\ cis}}\right) \quad (I)$$

15   The terms "trans" and "cis" are used to identify the
compartments on either side of the membrane.

The experiments illustrated in Figure 1A show the
reversal potential to be 35 mV for each filter, indicating
a selectivity value for cations over anions (t_+) of 0.93.

20   The relative pore sizes of the two filters are
estimated by assessing the effect on conductivity of
solutions of a non-electrolyte, polyethylene glycol (PEG),
of varying molecular weight. If the non-electrolyte is
able to pass through the pore, conductivity is decreased;

25 if not, it is unaffected. By determining the size of the
non-electrolyte that is unable to pass through the filter,
an estimate of its average pore size can be obtained.

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The conductivity of the pore in the presence or absence of 20% (v/v) polyethylene glycol (PEG) of differing molecular weights is compared. The conductances (G) are calculated from the modal current of the highest conducting

5   state at a potential of 0.2V for each filter ,in the presence of 20% PEG relative to that in the absence of PEG. The conductances for filter 1($\square$) and filter 2($\bullet$) are shown in Figure 1B and expressed as set out in equation II as the ratio (A) of that parameter with the same parameter but

10   measured in the absence of the filter:

$$A = \frac{(G^{filter}/G^{filter \, + \, PEG}) - 1}{(G^{solvent}/G^{solvent \, + \, PEG}) - 1} \qquad (II)$$

The approximate size of PEG which results in "cut

15   off" is considered to be the size which yields a value of A of 0.5. This size was found at molecular weights of ~3000 for filter 1 and ~1000 for filter 2. Based on the "exclusion" volume of PEGs in water, these correspond to pore radii of about 1.4 nm for filter 1 and 1 nm for filter

20   2. It is seen that the pore in filter 2 appears to be somewhat narrower than that in filter 1, compatible with a lower conductance (Fig. 1A).

The dependence of pore conductance on ionic strength is shown in Figure 2 as a double logarithmic plot.

25   Identical solutions containing KCl at the concentration indicated in Figure 2 with 10^{-4} - 3 x 10^{-3}M EDTA and 0.005M Hepes at pH 11($\blacklozenge$), 8.3($\bullet$), 7.5($\triangle$) or 6.5($\blacksquare$) bathed each face of filter 2. Conductance (G) of the highest conducting

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state at an applied potential of 0.8V is shown. Similar

data were obtained at an applied potential of 0.2V, except

that at pH 6.5 the high conducting state was not observed.

At pH 11 conductance is almost independent of ionic

5   strength for current (I) $\leq$ 0.1. At lower pH values the

conductance has a linear dependence on I but with a slope

much less than unity. These results suggest that negative

charges either in the pore or close to its mouth exert

considerable influence on the conductance by causing the

10   local cation concentration greatly to exceed that in the

bulk solution. The change of slope over the range of pH

values tested suggests that the negative charges titrate

with an effective pK around neutrality. Current

fluctuations are maximal around this pH, with smaller,

15   fewer fluctuations above or below it [4]. Such an effect

has recently been observed with *S. aureus* α-toxin induced

pores across lipid bilayers also [5].

Current fluctuations across the filters were measured

and typical records of current are shown in Figure 3A.

20   Filter 1 was bathed in 0.1M KCl, 0.005 M Hepes pH 7.4

without (i) or with 3.10^{-4}M (ii) or 3.10^{-3}M $CaCl_2$ (iii);

filter 2 (iv) was bathed in a similar medium without $CaCl_2$

at pH 8.0; currents at 0.2V were recorded on videotape

using a Biologic PCM Instrumentation Recorder and

25   subsequently analysed using Cambridge Electronic Design

Patch and Voltage-Clamp software. Figure 3A shows

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representative traces. Discrete changes between conducting

and non-conducting states are seen.

Analysis of the two predominant states (Figure 3B)

shows the higher one to be 11pA and the lower one 2.5pA for

5   filter 1. For filter 2 the values are 3.3pA and 1.2pA. The

lower state is unlikely to represent a "leak" current, as

(a) it changes on the addition of Ca^{2+} (see below), (b) it

disappears altogether at low pH and (c) it is not seen if

the filter is moved so that there is no pore at all between

10   the two chambers.

Figure 3C presents time distribution histograms for

the high and low conducting states respectively together

with the time constants for each state obtained by fitting

a single exponential to the distribution. For filter 1 mean

15   values (± S.D.) of the time constant for the high

conducting state (τ_H) of 210 ± 59 ms and of the low

conducting state (τ_L) of 23 ± 8.7 ms   (n = 8) were found.

The difference between the high and low conductance

states varies with voltage and ionic strength.

20   The amplitude of the difference between the current

of the high and low conducting states as a function of

applied voltage or of the ionic concentration of the

bathing medium are shown in table 1 below; n.d. indicates a

value was not determined.

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TABLE 1

Difference in current between the high and low conducting states of filter 2 at different potentials and ionic strengths.

Applied potential/V	current/pA	
	0.1M KCl	0.2M KCl
0.2	2.7	4.0
0.4	5.5	7.0
0.6	8.8	n.d.

Although the absolute value of the higher conductance state depends somewhat on the pre-treatment of the filter prior to use (e.g. exposure to ethanol, lipids, other chemicals, etc) the difference in amplitude between the higher conductance state and the lower one is remarkably constant. Other factors, such as pH (Figure 2) or the presence of divalent cations (see below) do affect both conductance states. On the addition of 0.3 mM Ca^{2+}, for example, the difference between the higher and lower conducting state is decreased to 9.5 and 3.5 pA respectively for filter 1 (Figure 3B). Such intermediate amplitudes also become detectable in the absence of Ca^{2+} if a long enough recording is analysed. Addition of 3 mM Ca^{2+} abolishes the high conducting state completely; high conductance is restored by the addition of EDTA (not shown). Similar effects are observed by addition of other

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divalent cations such as Zn^{2+} or Mg^{2+}, or by reduction of pH.

The relative efficacy of divalent cations and protons at reducing current is shown in Figure 4.   Filter 1 was

5   bathed in 0.1M KCl (Figure 4A) or 3M KCl (Figure 4B) with 0.005M Hepes, initial pH 7.4, H^+/OH^-(o) or divalent cations (at pH 7.4: Zn^{2+}($\triangle$), Ca^{2+}($\blacksquare$), Mg^{2+}($\blacklozenge$)) were added to give the final concentrations indicated. The time-averaged conductance (G) at 0.2V is expressed relative to the

10   maximum observed conductance (G_0) at the start of each titration. Figure 4C presents the logarithm of the ratio of the time constants for the high (τ_H) and low (τ_L) conducting states obtained at 0.2V in 0.1M KCl, 0.005M Hepes at the pH specified ($\square$ - filter 1; o - filter 2) or

15   in a similar medium at pH 7.4 (filter 1) containing the concentration of $CaCl_2$ ($\blacksquare$) or $ZnSO_4$ ($\triangle$) indicated; error bars represent the standard deviation found for filter 1 (n = 8) at pH 7.4 and for filter 2 (n = 4) at pH 8.0.

It is seen from Figures 4A and 4B that in both cases

20     $H^+ > Zn^{2+} > Ca^{2+} \geq Mg^{2+}$ with 50% inhibition at molar concentrations of approximately $10^{-7.7}$, $10^{-4.5}$, $10^{-3.4}$, and $10^{-3.0}$ (0.1M KCl) and $10^{-7.5}$, $10^{-2.8}$, $10^{-1.7}$ and $10^{-1.7}$ (3M KCl) respectively for filter 1, and $10^{-8.4}$, $10^{-6.0}$, $10^{-4.2}$, and $10^{-4.2}$ (0.1M KCl) for filter 2 (not shown). The fact that

25   inhibition is little different in 0.1M and 3M KCl suggests that simple screening of surface charge plays at best a minor role. The data points in Figure 4A and 4B are derived

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from time-averaged conductances. Kinetic analysis (as in
Figure 3C) of fluctuations observed in the presence of
divalent cations and protons is shown in Figure 4C. Both
sets of data show that filter 2 is more sensitive to
5   inhibition by divalent cations and protons, again
compatible with a smaller apparent pore size (see Figure
1B).

Ionic conduction in narrow pores may well be similar
to that which occurs in some hydrated zeolites where it can
10   be shown [6] that only the bare ion moves, although it is
at all times hydrated by an essentially static array of
water molecules. Calculation [7] shows that the centres of
the planar pentagon and puckered hexagon water rings that
preserve tetrahedral bonding can provide low energy ion
15   binding sites. The origin of the rapid switching maybe
connected with the fact that lattice sums [8] and molecular
dynamic simulations [9] show that water in narrow pores
will be electrically ordered. Changes in order could lead
to switching [8] due to the powerful electric fields
20   generated.

An alternative explanation of the switching is that
changes in the nature of the ions absorbed on the pore
walls alter the water structure near the pore walls and
hence the pore conductance. In zeolites it is known [10]
25   that exchange between cations in the bathing solution and
the alumina-silicate cage can lead to phase changes in the
cages and hence of the water contained within them. Such

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metastability may account for rapid transitions in the
conductance through narrow pores. In a biological system
the channel protein must also supply a gating mechanism
sensitive to the membrane voltage [11] and to the presence

5   of particular ligands [2], which may be in series with the
water-pore gating described here. In addition the protein
must distinguish between different cations [12] and carry
out other activities such as inactivation [13].

EXAMPLE 2

10   Individual pores in track-etched PETP filters
containing up to 5 x 10^9 pores cm^{-2} also exhibit selective
ion flow and rapid switching between discrete conductance
states provided that their overall ionic conductivity is
sufficiently low (less than 100pS in 0.1M KCl).

15   Such pores can be studied with glass micropipettes
(1) having a tip diameter of about 1μm using the apparatus
illustrated in Figure 5; ion currents through the pores in
the filter (between bath and pipette) are monitored with
Ag/AgCl electrodes in the electronic circuit shown. When

20   bath and pipette each contain 1M KCl with 0.005M Hepes, pH
adjusted to 7.4 with KOH the conductance recorded each time
the pipette contacts the filter falls in the range 5 to
2000pS as shown in Figure 6 (left hand panel). Assuming
that each conductance represents that through a cylindrical

25   pore in a 10μm thick filter, the diameter of that pore can
be calculated and the results of such calculations are
shown in Figure 6, right hand panel.   Pore diameters

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exhibit an approximately normal distribution with a mean
close to 200Å.

    Rapid switching between high and low conducting
states is observed when the bath and pipette contain 0.1M
5   KCl with 0.005M Hepes, pH adjusted to 7.4 with KOH and the
overall pore conductance does not exceed 100pS. Typical
results at positive or negative applied potentials are
shown for four different pores in Figure 7.

    Selectivity of ion currents through individual pores
10 using the apparatus shown in Figure 5 was assessed from the
reversal potential (the applied potential at which no ion
current flows) observed when the pipette contained 1M and
the bath 0.2M KCl each with 0.005M Hepes, pH adjusted to
7.4 with KOH. Cation selectivity, indicated by transference
15 numbers (t_+) in excess of 0.5, is observed for pores in
track-etched PETP filters whereas anion selectivity (t_+
less than 0.5) is observed in similar filters coated with
polyvinylmethylpyridine (PVMP) to confer positive surface
charge on the PETP. In either case pores with conductances
20 greater than 1000pS show little selectivity ($t_+ \approx 0.5$);
selectivity increases, for cations with PETP filters and
for anions with PVMP coated filters, progressively as pore
conductance diminishes (Figure 8).

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REFERENCES:

1.   Neher E and Sakmann B Nature $\underline{260}$, 799-802 (1976).

2.   Hille B. Ionic Channels of Excitable Membranes.
      Sinauer Associates Inc., Sunderland, Mass (1984) pp
      277, 303-353.

3.   Apel PY, Didyk A, Kravels LI and Kuznetsov VI Nucl.
      Tracks Radiat. Meas. $\underline{17}$ 191-193 (1990).

4.   Pasternak CA, Bashford CL, Korchev YE, Rostovtseva TK
      and Lev AA. (1993) Colloids and Surfaces A:
      Physicochemical Engineering Aspects $\underline{77}$ 119-124.

5.   Bezrukov S.M. and Kasianowicz JJ Bull. Am. Phys. Soc.
      $\underline{37}$, 625 (1992).

6.   Barrer RM and Rees LVC. Trans Faraday Soc. $\underline{56}$ 709-721
      (1960).

7.   Edmonds DT, Proc. R. Soc. B. $\underline{211}$ 51-62 (1980).

8.   Edmonds DT. In: Biological Membranes Vol. 5 (Ed D
      Chapman) Academic Press Inc. pp 341-379 (1984).

9.   MacKay DHC, Berens PH and Wilson KR. Biophys. J. $\underline{46}$,
      229-248 (1984).

10.   Barrer RM and Hinds L. J. Chem. Soc. 1879-1888
      (1953).

11.   Papazian DM, Timpe LC, Jan YN and Jan LY. Nature $\underline{349}$
      305-310 (1991).

12.   Yool AJ and Schwarz TJ, Nature $\underline{349}$ 700-704 (1991).

13.   Stuhmer W, Conti F, Suzuki H, Wang X, Noda M, Yahagi
      N, Kubo H and Numa S. Nature $\underline{339}$ 597-603.

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CLAIMS

1.   A process for controlling permeability to ions
and uncharged molecules through a pore as defined herein
having discrete states of differing permeability and being
5   of less than 10 nanometers radius or 20 nanometers width,
which process comprises placing the pore between a first
solution containing an ion (the transport ion) or uncharged
molecule capable of passing through the pore, and a second
solution;

10   and controlling the permeability of ions or uncharged
molecules through the pore by means of the transport ion
concentration in the first solution and/or the proton or
multivalent ion concentration in the first and/or second
solution.

15   2.   A process according to claim 1 in which the
pore has been created through a synthetic membrane by a
track-etch procedure.

3.   A process according to claim 2 in which the
pore is through a polyester membrane and is of less than 10
20   nanometers radius.

4.   A process according to claim 1 in which the
pore comprises two apposed synthetic membranes less than 20
nanometers apart.

5.   A process according to claim 4 in which the
25   apposed membranes have been etched.

6.   A process according to claim 1 where the first
solution is salinated water whereby differential flow of

- 22 -

ions through the pore causes desalination of the water, the
desalination being controlled by means of the concentration
of protons or divalent cations in the first solution.

7.   A switch for ionic current comprising a pore of
5   less than 10 nm radius through a synthetic membrane film or
comprising two apposed synthetic membranes less than 20 nm
apart forming a pore between the membranes, said pore being
simultaneously in contact with a first ionic solution
containing an ion capable of being transported through the
10 pore, the transport ion, and with a second ionic solution,

such that when the switch is placed in an electric
circuit, flow of ionic current though the pore is
controlled by transport ion concentration in the first
ionic solution and/or the proton or multivalent ion
15 concentration in one or both of the said ionic solutions.

8.   A switch as defined in claim 7 in which the
pore is as defined in claim 2, 3, 4 or 5.

9.   A sensor for sensing a parameter in a solution,
said sensor comprising a pore of less than 10 nanometers
20 radius through a synthetic membrane or comprising two
apposed synthetic membranes less than 20 nm apart forming a
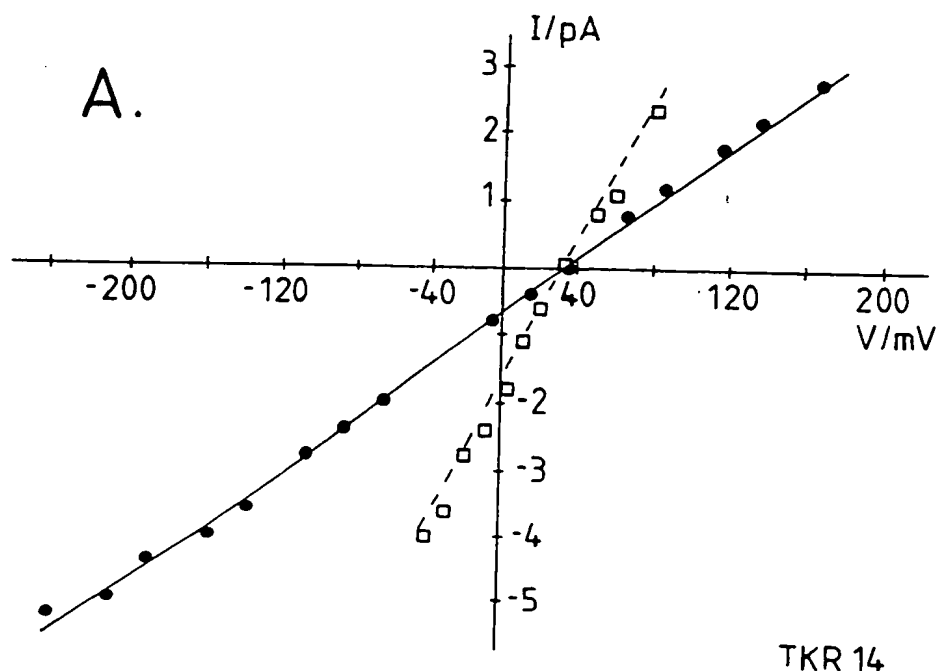pore between the membranes,

such that when the pore is placed in contact with the
solution whose parameter is to be detected, the ionic
25 current passing through the pore is indicative of the
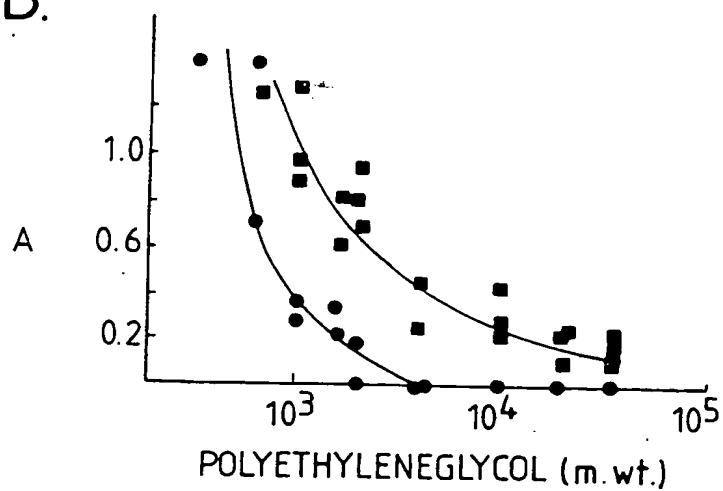presence, absence or level of the parameter to be detected.

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10.   A sensor as defined in claim 9 in which the pore is as defined in claim 2, 3, 4 or 5.

Fig.1.

A.

TKR 14

B.

POLYETHYLENEGLYCOL (m.wt.)

TKR 13

SUBSTITUTE SHEET (RULE 26)
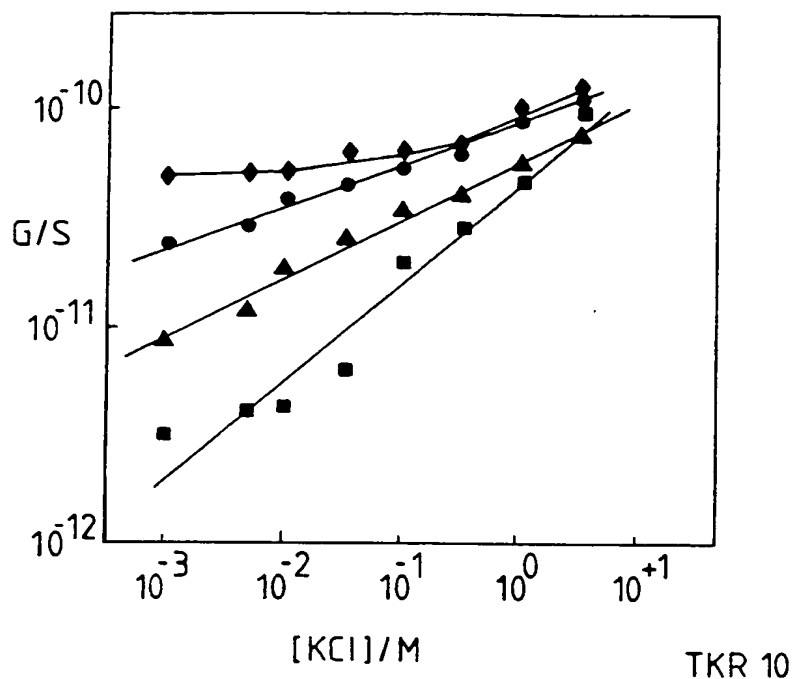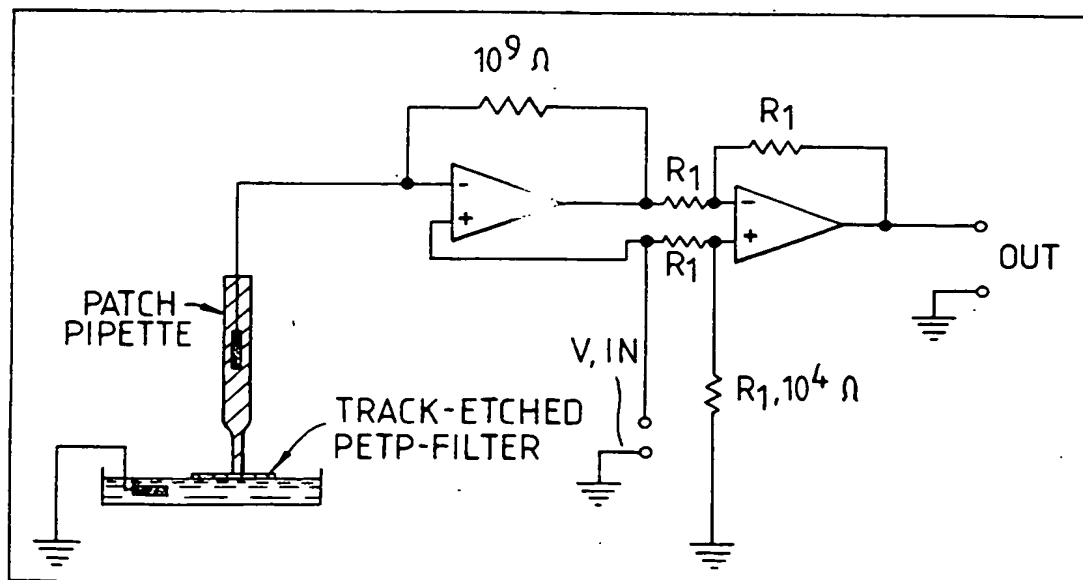
Fig.2.

G/S

[KCl]/M

TKR 10

Fig.5.

APPARATUS TO MONITOR ION CURRENTS IN A TRACK-ETCHED,
POLYETHYLENE TEREPHTHALATE FILTER.
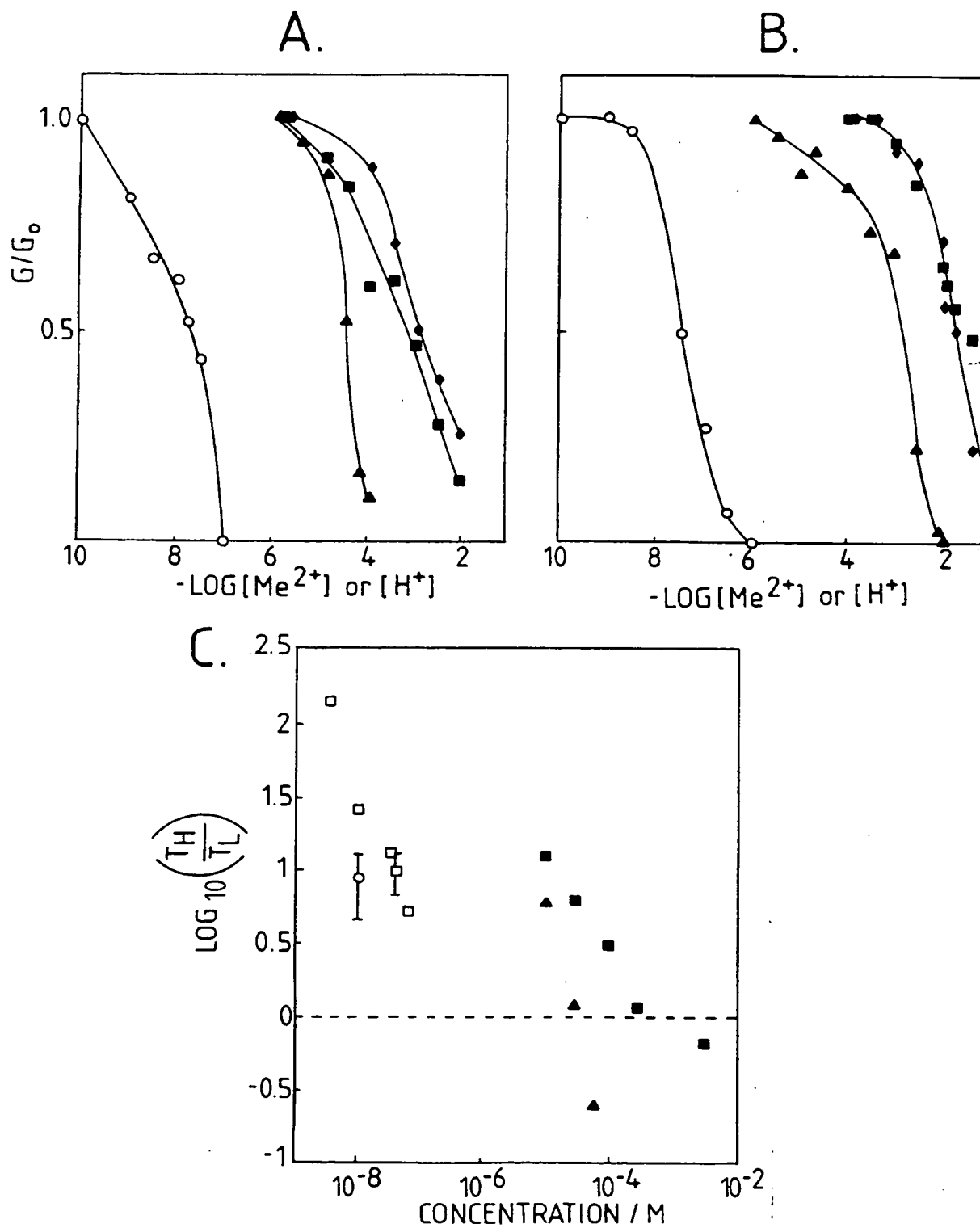
BNSDOCID: <WO___9415701A1_I_>

Fig.3.

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Fig. 4.
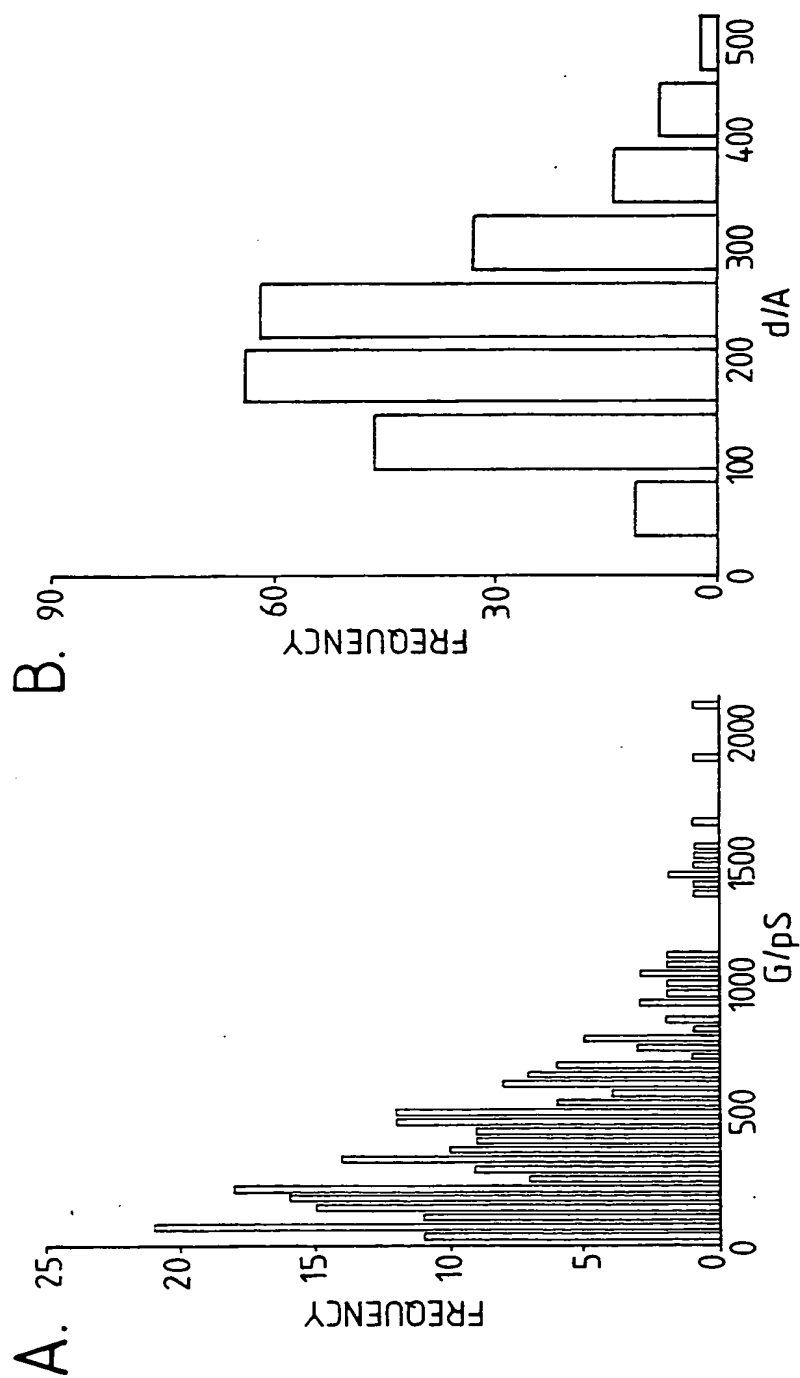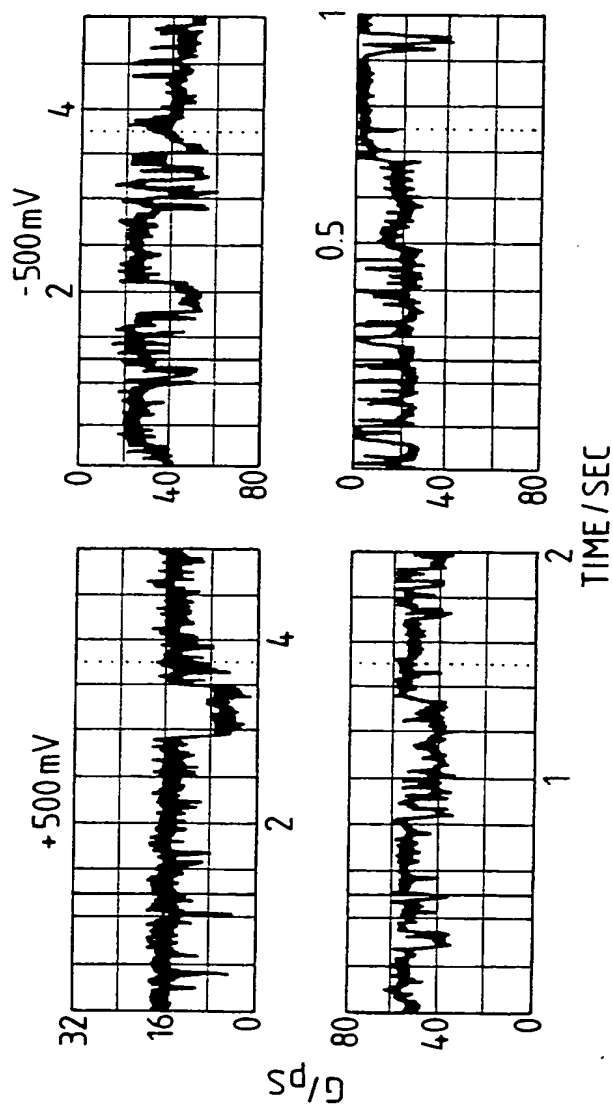
Fig.6.

FREQUENCY DISTRIBUTION OF IONIC CONDUCTIVITY (A) AND CALCULATED DIAMETER (B) OF PORES IN A TRACK - ETCHED, PETP - FILTER.

FILTERS (1.4×10^9 PORES/cm^2), 1.0M KC1, 0.005M HEPES pH 7.4 IN BATH AND PIPETTE

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Fig.7.

PATCH - PIPETTE CURRENTS FROM A TRACK - ETCHED, PETP - FILTER FILTER (1.4 x 10⁹ PORES/cm²) ON 0.1M KC1, 0.005M HEPES, pH 7.4 PIPETTE (~1 μm DIAMETER) FILLED WITH A SIMILAR SOLUTION.
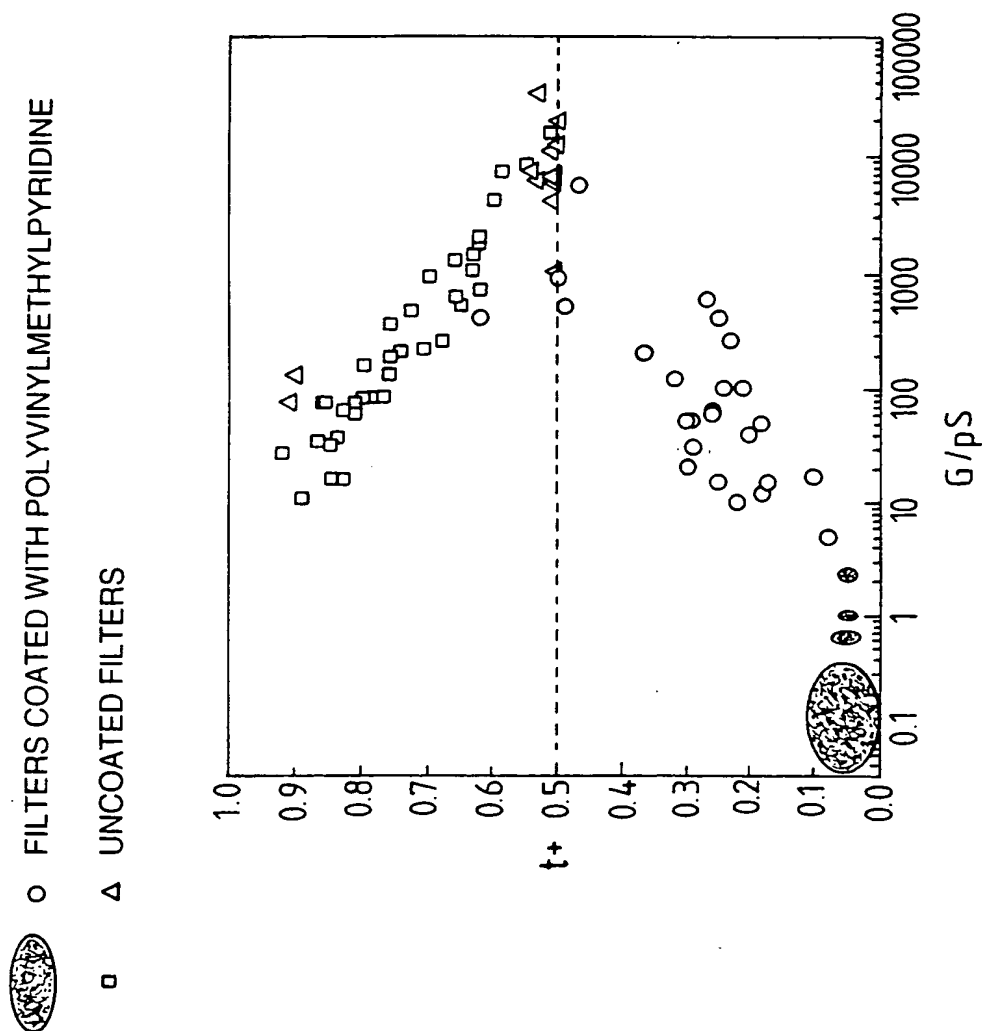
Fig. 8.

IONIC SELECTIVITY OF PORES IN TRACK - ETCHED, PETP - FILTERS
1.0M KCl (PIPETTE)/0.2MKCl (BATH), 0.005M HEPES, pH 7.4

o   FILTERS COATED WITH POLYVINYLMETHYLPYRIDINE

△   UNCOATED FILTERS

□

BNSDOCID: <WO___9415701A1_I_>

International Application No.

PCT/GB 94/00032

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5   B01D61/00   B01D69/02   B01D61/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5   B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	EP,A,0 562 303 (GESELLSCHAFT FÜR SCHWERIONENFORSCHUNG MBH) 29 September 1993 see the whole document ---	1
A	DE,A,38 31 970 (J. HEITBAUM ET AL.) 22 March 1990 ---	
A	WO,A,90 08783 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH INSTITUTE) 9 August 1990 ---	
A	US,A,3 097 076 (A. F. REID) 9 July 1963 ---	
	-/--	

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

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Date of the actual completion of the international search

11 April 1994

Date of mailing of the international search report

27. 04. 94.

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	C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF MEMBRANE SCIENCE vol. 68, no. 3 , 15 April 1992 pages 263 - 270 N. N. SUDAREVA ET AL. 'INCREASE IN EFFICIENCY OF MEMBRANE FRACTIONATION' ---	
A	ENVIRONMENTAL SCIENCE & TECHNOLOGY vol. 26, no. 3 , March 1992 pages 569 - 577 J. KILDUFF ET AL. 'TRANSPORT AND SEPARATION OF ORGANIC MACROMOLECULES IN ULTRAFILTRATION PROCESSES' -----	

1

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0562303	29-09-93	DE-A-	4305979	23-09-93
DE-A-3831970	22-03-90	NONE		
WO-A-9008783	09-08-90	AU-B-	623747	21-05-92
		AU-A-	5033490	24-08-90
		EP-A-	0455705	13-11-91
		JP-T-	4504714	20-08-92
US-A-3097076		NONE		

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